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ON

EXPERIMENTAL STUDIES FOR THE DETECTION OF PROTEIN IN TRACE AMOUNTS (J-BANDS)

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The influence of the following biological and synthetic materials on the absorption spectrum of aqueous solutions of the dye 3,3' diethyl-9-methyl 4,5,4',5' dibenzothiacarbocyanine bromide (I) were determined:

a. Proteins

Thirty-six proteins dissolved in cacodylic acid buffer (pH 7.0) and distilled water were investigated. The results obtained were quite variable, but only in the case of hemoglobin, carboxypeptidase, insulin, and beta lipoprotein did the proteins fail to influence the absorption spectrum of the dye. In general, the changes which occurred could be attributed to various combinations of increases in absorbance at about 535, 570, and 650 mμ. Increases in absorbance at the following combinations of wavelengths were observed: (1) 535, 570, or 650 mμ only, (2) 570 plus 650 mμ, (3) 570 plus 535 mμ, and (4) 570 plus 535 plus 650 mμ.

b. Di- and Tripeptides

Forty peptides dissolved in cacodylic acid buffer and distilled water were investigated. Only six of these peptides produced changes in the dye spectrum at a concentration of 0.02 percent or less. At higher concentrations (0.1 percent) most of the peptides caused alterations in the absorption spectrum of the dye. The alterations, as in the case of proteins, generally involved combinations of increases in absorbance at wavelengths of about 535, 570, and 650 mμ. The reactions obtained were analyzed on the bases of: (1) the occurrence of hydrophobic, hydrophilic nonionizable, and hydrophilic ionizable side-chains present in the peptides, (2) the presence of specific amino acids, and (3) the amino acid sequence in the peptide chain. The hydrophilic or hydrophobic nature of the side chains did not appear to have any consistent influence upon the nature of the changes in the absorption spectrum. The sequence of the amino acids in the peptide was of considerable importance; e.g., leucyl glycine caused the formation of an intense J-band at 650 mμ, whereas glycyl leucine was without effect and glycyl alanine induced a new absorption peak at 540 mμ, but alanyl glycine did not influence the absorption spectrum. The presence of a specific terminal amino acid did not determine the nature of the change in the absorption spectrum of the dye. In fact, the nature of the change induced depended upon the specific amino acid combinations in the peptide.

c. Synthetic Polypeptides

Poly L lysine hydrobromide, poly L hydroxyproline, and poly L aspartic acid dissolved in cacodylic acid buffer (pH 7.0) were examined. A 0.02 percent solution of poly L hydroxyproline did not alter the absorption spectrum of the dye, whereas a 0.02-percent solution of poly L lysine hydrobromide caused the formation of a small J-band and a 0.0001 percent solution of poly L aspartic acid produced a large absorption peak at 550 mμ, but no J-band. Apparently, the presence of a compound with regularly spaced, ionizable, anionic, side chains is especially conducive to the induction of the absorption peak in the 540 mμ region, whereas regularly spaced ionizable cationic side chains are not.

d. Deoxyribonucleic (DNA) and Acid and Ribonucleic Acid (RNA)

Interaction of DNA or RNA with dye I did not cause the formation of J-bands. However, new intense absorption peaks were found at 535 mμ in the RNA solution and at about 570 mμ in the DNA solution. The absorption peak in the DNA solution was readily evident with solutions containing only 0.0002 percent DNA.

e. Carbohydrates

The influence of 0.02 percent solutions of mono-, di-, tri-, and long-chain polysaccharides dissolved in cacodylic acid buffer and water on the absorption spectrum of the dye was determined. Forty-two carbohydrates were tested. The mono-, di-, and trisaccharides did not alter the absorption spectrum of the dye in any manner, but some of the polysaccharides caused marked changes. Insulin and dextrin had no effect, but xylan, heparin, polygalacturonic acid, agar, chondroitin sulfate, algenic acid, hyaluronic acid, and carboxymethyl cellulose ether altered the spectrum considerably. Each of these substances affected the dye spectrum in a different way, but in each case, one and sometimes two new absorption peaks appeared at longer wave lengths than the dye peak. These new peaks were found between 520 and 640 mμ, and they appeared in the presence of as little as 0.0002 to 0.00006 percent of the substance tested.

The relationship of the isoelectric point of proteins to their ability to induce new absorption peaks in dye I was examined by observing the intensity of the induced peak as a function of pH. In every case, a decrease in pH resulted in a decrease in the intensity of the induced absorption peak and quite often there was a critical pH at which the ability of the protein to induce new absorption peaks was drastically decreased. Except in the case of gelatine, this critical pH did not correspond to the published isoelectric point of the protein, and there appeared to be no relationship between the isoelectric point of the protein and the pH at which the protein induced new absorption peaks.

Gelatine, beta lactoglobulin, carbonic anhydrase, myoglobin, KH_2PO_4, carboxypeptidases lysozyme and DNA, all of which cause large changes in the absorption spectrum of dye I, were examined to determine the effect of the length of the reaction and the temperature of reaction on the intensity of induced absorption peaks. At room temperature, the maximum intensity was reached within 80 minutes after mixing the dye and the test substance. The effect of temperature on the reactions was especially significant. Induction of J-bands by inorganic salts was favored by temperatures of less than 20°C. At 7°C a very intense J-band was formed in the presence of KH_2PO_4. With increasing temperature, the intensity of the J-band diminished, and at 31°C the absorption spectrum of the dye was not affected by the KH_2PO_4. On the other hand, the formation of J-bands in the presence of proteins is favored by higher temperatures. For example, with 0.005 percent beta lactoglobulin, the intensity of the J-band at 40°C is six times greater than at 5°C. In contrast to inorganic salts and proteins, the intensity of the 570 mμ absorption peak obtained with DNA does not appear to be significantly altered by temperature changes over the range 7 to 66°C.

The reaction of dye I with samples of a relatively non-fertile, sandy soil was investigated. One gram of this soil was extracted with 10 ml of distilled water, and an aliquot of the supernate was mixed directly with the dye or dialyzed against distilled water, to remove small molecules, and then mixed with the dye. Large increases in absorbance in the 640 mμ region of the spectrum were observed, and an intense new absorption peak was formed at 530 mμ in both the dialyzed and untreated samples. Control experiments with added inorganic salts indicated that the dialysis was sufficient to remove small molecules. Therefore, the changes in the absorption spectrum were due to macromolecules. Soil samples were also freed of organic matter by incineration. When the supernate from mixtures of the organic-free soil and water were added to the dye solution, no changes in the absorption spectrum of the dye were evident.

The dye 3,3' dimethyl 5,5' dichloro-9-ethyl thiacarbocyanine chloride was examined for its reaction with proteins, nucleic acids, and inorganic salts. Reactions similar to those with dye I were found. However, the use of this dye in preference to dye I does not appear to be advisable since it is less sensitive and exhibits multiple absorption peaks in aqueous solution which add to the difficulties of interpretation.

The results obtained in this quarter appear to be especially encouraging for the possible application of the dye reaction to the detection of trace amounts of macromolecules in heterogeneous samples. The reactions obtained continue to show very high sensitivities, and they exhibit special characteristics which may yield considerable information about the nature of the macromolecules. The method appears to be suitable for the detection of most proteins and, in addition, the dye exhibits rather specific and sensitive reactions with nucleic acids and long-chain polysaccharides. One of the most important findings is the difference in the manner in which temperature influences the reaction of the dye in the presence of nucleic acids, proteins, and inorganic salts. This difference may present an exceptionally easy method, aside from the differences in the position and nature of the absorption peaks, for distinguishing among proteins, nucleic acids, and inorganic salts. By simply determining the optical density of the preparation at two temperatures, for example 10- and 30°C, an immediate distinction can be made; an increase in temperature will decrease the response due to inorganic salts, accentuate the response caused by proteins, and not affect changes induced by nucleic acids.